ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Genetic Diversity in Aromatic Rice (*Oryza sativa* L.) Genotypes using Microsatellite Markers

Neha Agrawal*and Rajeev Shrivastava

Department of Genetics and Plant Breeding, IGKVV, Raipur, Chhattisgarh

*Corresponding author-pnehaagrawal@gmail.com

Received: 30th December, 2014; Accepted: 10th May, 2015

Abstract

The genetic diversity and DNA fingerprinting of 40 aromatic rice genotypes constituting landraces collected from different regions of Chhattisgarh were assessed using 21 microsatellite markers distributed over 12 chromosome of rice. The results revealed that all the SSRs were polymorphic with different alleles among the cultivars studied indicating the robust nature of microsatellites in revealing polymorphism. A total of 50 alleles were amplified among the 21 SSRs. Hundred per cent similarity was observed between genotypes Jeeradhan and Jawaphool; Bisni-I and Bisni –III. Principal component analysis was done to visualize genetic relationships among the aromatic rice landraces. The information obtained from the DNA fingerprinting studies helps to distinctly identify and characterize 40 aromatic rice landraces using 21 different RM primers. A basic molecular database created for aromatic landraces of rice will be useful for future reference and to protect this unique rice under IPR regime. This information can be used in background selections during backcross breeding programs.

Key words: Genetic diversity, microsatellite marker, dendogram.

Introduction

Rice (*Oryza sativa* L.) is one of the most important crops that provide food for more than half of the world population. India has a long history of rice cultivation and stands first in rice area and second in rice production, after China. Chhattisgarh is very rich for biodiversity but the resources are not yet properly utilized. This geographical region has vast diversity of rice. Some of the local rice or land races having unique identity and taste are very much in demand by traders and consumers. The research efforts have been focused to develop high yielding dwarf rice varieties having resistance to biotic stresses but less emphasis has been given to improve the local aromatic rice of Chhattisgarh. Though the land races are being maintained by the farmers traditionally, they are not released as varieties and are not notified and not in seed production chain.

Rice is also a model crop for the study of genetics and genome organization due to its diploid genetics, relatively small genome size (430 Mb) (Causse *et al*., 1994; Kurata *et al.,* 1994) and significant level of genetic polymorphism (McCouch *et al.,* 1998; Tanksley, 1983, Wang *et al.,* 1992). Scope of crop improvement depends on the conserved use of genetic variability and diversity in plant breeding programmes and also with the use of molecular markers. A large amount of well conserved genetically diverse material (approximately 23250 accessions of rice germplasm) is available at Indira Gandhi Krishi Vishwavidyalay, Raipur, Chhattisgarh.

Characterization and quantification of genetic diversity has long been a major goal in evolutionary biology. Information on the genetic diversity within and among closely related crop varieties is essential for a rational use of genetic resources. The analysis of genetic variation both within and among elite breeding materials is of fundamental interest to plant breeders. It contributes to monitoring germplasm and can also be used to predict potential genetic gains. Diversity based on physiological and morphological characters usually varies with environments and evaluation of these traits requires growing the plants to full maturity prior to identification. The rapid development of biotechnology allows easy analysis of a large number of loci distributed throughout the genome of plants.

Molecular markers have proven to be powerful tools in the assessment of genetic variation and in the elucidation of genetic relationships within and among species. Several molecular markers *viz.,* RFLP (Becker *et al.,* 1995; Paran and Michelmore, 1993;), RAPD (Tingey and Deltufo, 1993; Williams *et al.,* 1990), SSRs (Levinson and Gutman, 1987), ISSRs (Albani and Wilkinson, 1998; Blair *et al.,* 1999), AFLP (Mackill *et al.,* 1996; Thomas *et al.,* 1995; Vos *et al.,* 1995; Zhu *et al*., 1998) and SNPs (Vieux *et al*., 2002) are presently available to assess the variability and diversity at molecular level (Joshi *et al.,* 2000). Information regarding genetic variability at molecular level could be used to help, identify and develop genetically unique

germplasm that compliments existing cultivars. In the present investigation, assessment of genetic variability and diversity at molecular level among 40 aromatic rice genotypes was assessed using 21 SSR markers spanning 12 chromosomes.

Materials and Methods

Forty aromatic rice landraces from different parts of Chhattisgarh were collected *viz*., Jeeradhan, Jawaphool (Raigarh), Dubraj-II (Dhamtari), Mai dubraj, Chinnor, Karigilas, Dubraj-I (Nagri Sihwa), Dubraj-III, Dujai (Pendra), Dubraj-IV (Bemetra), Kasturi, Anterved, Lallu-14, Kapoorsar, Badhsaahbhog (Jagdalpur), Kalikamod, Gangabaru (Bastar), Jeeraphool (Ambikapur), Aatmasheetal (Bastar), Chhatri, Shyamjeera (Surajpur), Kubrimohar (Bemetra), Gopalbhog, Shuklaphool, Tilkasturi, Samudrafan, Bisni-II, Bisni-I, Bisni-III, Lohndi (Garola), Jaigundi, Dubraj-V (Kharora), Keragul, Jaophool, Katarnibhog, Srikamal, Tulsiprasad, Tulsimanjari, Elayachi and Vishnubhog. These genotypes are very popular among consumers due to their unique aroma and taste.

Plant materials and genomic DNA isolation

Fourty aromatic landraces of rice were planted in nursery bed, after 2 weeks of sowing about one gram leaf of seedlings were collected and DNA was isolated from these leaves by using mini prep method of DNA extraction (Dellaporta *et al*., 1983). The DNA samples were quantified on Nano Drop Spectrophotoscopy (NANODROP 2000). After quantification, the DNA was diluted with TE such that the final concentration of DNA was approximately 40-50 ηg/µl for PCR amplification.

PCR amplification and electrophoresis

A set of 21 microsatellite markers distributed over 12 chromosomes of rice were used. 2 ml of diluted template DNA of each genotype was dispensed at the bottom of PCR plate (OXYGEN). Separately cocktail was prepared in an Eppendorf tube as described in Table 1. About 18ml of cocktail was added to each sample and the PCR (Life Technologies, Applied Bio system Ltd) was set up as the profile depicted in Table 2. Five per cent polyacrylamide gels (vertical) were used for better separation and visualization of PCR amplified products, since polyacrylamide gel (PAGE) have better resolution for amplified products.

Results and Discussion

Assessment of genetic diversity is an essential component in germplasm characterization and conservation. Selection increases the frequency of alleles or allelic combinations with favorable effects at the expense of others, eventually eliminating many of them (Cao *et al*., 1998). In the present investigation microsatellites (Rice microsatellites) or SSR markers (Simple Sequence Repeats) were used to characterize and assess genetic diversity among 40 aromatic rice land races of Chhattisgarh. A total of 21 RM primers were utilized to provide genetic diversity among 40 aromatic rice landraces. Eighteen RM primers showed polymorphism in these cultivars.

Many studies have also reported significantly greater allelic diversity of microsatellite markers than other molecular markers (Mc Couch *et. al*., 2001). Rice similarity ratio revealed that high degree of similarity to the extent of 100 per cent exists between Jeeradhan and Jawaphool, and Bisni-I and Bisni-III, lowest level of similarity of 54 per cent exists between Elaychi and Visnubhog. It is important to note here that Bisni-I and Bisni-III are the sister lines selections and though Jeeradhan and Jawaphool were collected from fields of different farmers but from the same village. Highest similarity co-efficient among them indicated similar genetic background. Whereas, Elaychi land race showed different plant characters with all these lines and Vishnubhog also showed least similarity with rest of the entries. Similar studies were made by different authors using SSR markers (Panaud *et al*., 1996; Chakravarthi and Naravaneni, 2006.).

The UPGMA cluster analysis was performed by using Jaccard's similarity coefficient matrix prepared by binary score generated by using 21 microsatellite markers situated on different chromosomes of rice. The similarity coefficient ranged from 43-100 per cent. Two major clusters were formed which exhibited 46 per cent genetic similarity. First cluster consisted of 13 genotypes, whereas second cluster consisted of 27 genotypes of aromatic rice.

Second cluster is further divided into two groups, among which genotype Elaychi and Vishnubhog included and these genotypes exhibited 54 per cent genotypic similarity. The other genotype of this cluster exhibited 56 per cent genetic similarities further divided into two groups of which one group consisted of 24 genotypes whereas Tulsimanjari alone formed a separate group.

In first cluster, two major groups were formed at 46 per cent similarity. 11 genotypes clustered in one in group and exhibited 62 per cent genetic similarity, whereas other group had two genotypes Anterved and lallu-14 with 66 per cent genetic similarity.

For the loci studied, genotypes Jeeradhan and Jawaphool exhibited 100 per cent similarity. In the accessions three of

them from Bisni (aromatic rice collected from Ambikapur and Bagicha Districts of Sarguja). Bisni-I and Bisni-III also exhibited 100 per cent genetic similarity, but with Bisni-II these two showed 90 per cent genetic similarity these all 3 lies in 2nd cluster. Similarly five different accessions of Dubraj were also studied for SSR markers. Out of which Dubraj-II and Maidubraj exhibited 95 per cent genetic similarity. Dubraj-I and Dubraj-III has 88 per cent genetic similarity. Between Dubraj-I and III and Dubraj-IV 67 per cent genetic similarity was recorded. An accession of Dubraj-V recently collected from farmers laid on 2nd cluster exhibited that between Dubraj-5 and rest of the Dubraj accessions *i.e.,* Dubraj-I, II, III, IV, and Mai Dubraj only 43 per cent genetic similarity exists.

In second major cluster, two major groups were formed. Two entries of the second group *i.e.,* Elaychi and Vishnubhog have 53 per cent genetic similarity and with rest of the entries of the group these two showed 46 per cent genetic similarity. There results indicated that all the genotypes have high amount of genetic variability. It can be further utilized in breeding program for developing new varieties. Similar observations were made by Akagi *et al*. (1997).

Cluster analysis was used to group the varieties and to construct a dendrogram. This dendrogram revealed that the genotypes are derivatives of genetically similar type clustered more together. In this study, the larger range of similarity values for cultivars revealed by micro satellite markers provides greater confidence for the assessments of genetic diversity and relationships, which can be used in future breeding programs. Principle component analysis was also done to visualize genetic relationships among the elite breeding lines.

This fingerprinting makes identification and characterization of genotype very easy and further it will be of greater help in background selections during back cross breeding programmes.

References

Akagi, H., Yokozeki, Y., Inagaki, A. and Fujimura, T. 1997. Highly polymorphic microsatellites of rice consist of AT repeats, and a classification of closely related cultivars with these microsatellite loci. *Theoretical and Applied Genetics* 94:61-67.

Albani, M.C. and Wilkinson, M.J. 1998. Inter simple sequence repeat polymerase chain reaction for the detection of somaclonal variation. *Plant Breeding* 117:573-575.

Becker, J., Vos, P., Kuiper, M., Salamini, F. and Heun, M. 1995. Combined mapping of AFLP and RFLP markers in barley. *Mol. Gen. Genet*., 249: 65-73.

Blair, M.W., Panaud, O. and McCouch, S. R. 1999. Inter-simple sequence repeat (ISSR) amplification for analysis of microsatellite motif frequency and fingerprinting in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics* 98: 780-792.

Cao, T., Duprez, E., Borden, K.L.B., Freemont, P.S. and Etkin, L.D. 1998. Ret finger protein is a normal component of PML nuclear bodies and interacts directly with PML. *J. Cell Science* 111:1319-1329.

Causse, M.A., Fulton, T.M., Cho, Y.G., Ahn, S.N., Chunwongse, J., Wu, K., Xiao, J., Yu, Z., Ronald, P.C., Harrington, S.E., Second, G., McCouch, S.R. and Tanksley, S.D. 1994. Saturated molecular map of the rice genome based on a inter specific backcross population. *Genetics*138:1251-1274.

Chakravarthi B.K. and Naravaneni Rambabu. 2006. SSR marker based DNA fingerprinting and diversity study in rice (*Oryza sativa*. L). *African Journal of Biotechnology* 5 (9), pp. 684-688.

Dellaporta, S.L., Wood, J. and Hicks, J.B. 1983. A plant DNA mini preparation: version II. *Plant Molecular Biology*1:19-21.

Joshi, S.P., Gupta, V.S., Aggarwal, R.K., Ranjekar, P.K. and Brar, D.S. 2000. Genetic diversity and phylogenetic relationship as revealed by Inter simple sequence repeat polymorphism in the genus *Oryza*. *Theoretical and Applied Genetics* 100:1311-1320.

Kurata, N., Nagamura, Y., Yamamot, K., Harushima, Y., Sue, N., Wu, J., Antonio, B.A., Shomura, A., Shimizu, T. and Lin, S.Y. 1994. A 300 kilo base interval genetic map of rice including 883 expressed sequences. *Nature Genetics* 8:365-372.

Levinson, G. and Gutman, G.A. 1987. Slipped-strand mis-pairing: A major mechanism of DNA sequence evolution. *MolecularBiology Evolution* 4:203-221.

Mackill, D. J., Zhang, Z., Redona, E. D. and Colowit, P. M. 1996. Level of polymorphism and genetic mapping of AFLP markers in rice. *Genome* 39: 969-977.

McCouch, S.R., Kochert, G., Yu, Z.H., Wang, Z.Y. and Khush, G.S. 1988. Molecular mapping of rice chromosomes. *Theoretical and Applied Genetics*76: 815-829.

McCouch, S.R., Temnykh, S., Lukashova, A., Coburn, J., Declerck, G., Cartinhour, S., Harrington, S., Thomson, M., Septiningsi, E., Semon, M., Moncada, P. and Jiming, L. 2001. Microsatellite markers in rice: Abundance, diversity and applications. *In: Rice Genetics IV*. IRRI. Manila, Philippines. pp: 117-135.

Panaud, O., Chen, X. and McCouch 1996. Development of microsatellite markers and characterization of simple sequence length polymorphism (SSR) in rice (*Oryza sativa* L.). *Mol. Gen. Gene*t., 252: 597–607.

Paran, I. and Michelmore, R.W. 1993. Development of reliable PCR based markers to downy mildew resistance genes in Lettuce. *Theoretical and Applied Genetics* 85:985-993.

Tanksley, S.D. 1983. Molecular markers in plant breeding. Plant Molecular Biology Reports 1:3-8.

Thomas, C.M., Vos, P., Zabeau, M., Jones, D. A. and Norcottet, K.A. 1995. Identification of amplified restriction fragment polymorphism (AFLP) markers tightly linked to the tomato Cf-9 gene for resistance to Cladosporium fluvum. *Plant Journal* 8:785-794.

Tingey, S.V. and Deltufo, J.P. 1993. Genetic analysis with Random Amplified Polymorphic DNA. *Plant Physiology* 101:349-352.

Vieux, E.F., Kwok, P.Y. and Miller, R.D. 2002. Primer design for PCR and sequencing In:High-throughput analysis of SNPs. *Biotechniques* 32: 28-30.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee T, Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M. and Zabeau, M. 1995. AFLP: A new technique for DNA fingerprinting. *Nucleic Acids Research* 23: 4407- 4414.

Wang, Z.Y., Second, G. and Tanksley, S.D. 1992. Polymorphism and phylogenetic relationships among species in the genus *Oryza* as determined by analysis of nuclear RFLPs. *Theoretical and Applied Genetics* 83: 565-581.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A. and Tingey, S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* 18:6531-6535.

Zhu, J., Gale, M.D., Quarrie, S., Jackson M. T. and Bryan, G.J. 1998. AFLP markers for the study of rice biodiversity. *Theoretical and Applied Genetics* 96:602-611.

Table 1. PCR mix for one reaction

Reagent	Stock concentration	Volume (ml)
Sterile and nanopure H_2O	-	13.5
PCR buffer with 15 mM $MgCl_2$	10X	2.0
dNTPs (Mix)	1mM	1.0
Primer (forward+ reverse)	5ρmol	1.0
Taq polymerase	1 u/ml	0.5
DNA template	40 ηg/ml	2.0
	Total	20

Table 2. Temperature profile used for PCR amplification using micro-satellite markers

Steps	Temperature (°C)	Duration (min.)	Cycles	Activity
1	95	5	1	Denaturation
2	94	1		Denaturation
3	55	1	34	Annealing
4	72	1		Extension
5	72	7	1	Final Extension
6	4	∞	1	Storage

Table 3. SSR primers used to amplify the *O. sativa* in study

Chro. Numbers	SSR Primers	PRIMER SEQUENCES	
		FORWARD 5' → 3'	REVERSE 5' → 3'
1	RM 5	TGCAACTTCTAGCTGCTCGA	GCATCCGATCTTGATGGG
1	RM 431	TCCTGCGAACTGAAGAGTTG	AGAGCAAAACCCTGGTTCAC
2	RM 13541	CTCCTCGCTTCGTCCACTTCC	CCATGTGTCACCGACTCAACG
2	RM 7485	GCCAGTTTCTCCAAAAGACG	AACTAGCCTCGACAGCGAAC
3	RM 55	CCGTCGCCGTAGTAGAGAAG	TCCCGGTTATTTTAAGGCG
3	RM 514	AGATTGATCTCCCATTCCCC	CACGAGCATATTACTAGTGG
4	RM 307	GTACTACCGACCTACCGTTCAC	CTGCTATGCATGAACTGCTC
5	RM 507	CTTAAGCTCCAGCCGAAATG	CTCACCCTCATCATCGCC
5	RM 161	TGCAGATGAGAAGCGCCGCCTC	TGTGTCATCAGACGGCGCTCCG
6	RM 162	GCCAGCAAAACCAGGGATCCGG	CAAGGTCTTGTGCGGCTTGCGG
6	RM 454	CTCAAGCTTAGCTGCTGCTG	GTGATCAGTGCACCATAGCG
7	RM 455	AACAACCCACCACCTGTCTC	AGAAGGAAAAGGGCTCGATC
8	RM 44	ACGGGCAATCCGAACAACC	TCGGGAAAACCTACCCTACC
8	RM 408	CAACGAGCTAACTTCCGTCC	ACTGCTACTTGGGTAGCTGACC
9	RM 105	GTCGTCGACCCATCGGAGCCAC	TGGTCGAGGTGGGGATCGGGTC
9	RM 316	CTAGTTGGGCATACGATGGC	ACGCTTATATGTTACGTCAAC
10	RM484	TCTCCCTCCTCACCATTGTC	TGCTGCCCTCTCTCTCTCTC
11	RM 144	TGCCCTGGCGCAAATTTGATCC	GCTAGAGGAGATCAGATGGTAGTGCATG
11	RM 536	TCTCTCCTCTTGTTTGGCTC	ACACACCAACACGACCACAC
12	RM 1261	GTCCATGCCCAAGACAAC	GTTACATGGGTGACCCC
12	RM 277	CGGTCAAATCATCACCTGAC	CAAGGCTTGCAAGGGAAG

1. Jeeradhan
2. Jawaphool
3. Anterved
4. Kapoorsar
5. Badhsaahbhog
6. Dubraj-III
7. Aatmasheetal
8. Karigilas
9. Chhatri
10. Elayachi
11. Gopalbhog
12. Tilkasturi
13. Tulsimanjari
14. Maiubraj
15. Shuklaphool
16. Dubraj-I
17. Kalikamod
18. Dujai
19. Lallu-14
20. Gangabaru
21. Dubraj-IV
22. Dubraj-II
23. Jeeraphool
24. Kubrimohar
25. Jaophool
26. Katarnibhog
27. Shrikamal
28. Vishnubhog
29. Shyamjeera
30. Dubraj-V
31. Kasturi
32. Lohndi
33. Keragul
34. Chinnor
35. Samudrafen
36. Jaigundi
37. Tulsiprasad
38. Bisni-III
39. Bisni-II
40. Bisni-I